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Effects of quaternary ammonium ions on vasorelaxation induced by endothelial and exogenous nitric oxide

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Abstract

The present study was aimed to examine the effects of three quaternary ammonium ions on both endothelial and exogenous nitric oxide (NO)-mediated relaxation in the rat aorta and to examine whether L-arginine could antagonize the inhibitory effects on endothelial NO-dependent vascular responses. In endothelium-intact aortic rings, cyclopiazonic acid (CPA) induced relaxation with a pD_2 of 6.40 ± 0.06. This relaxation was attenuated after treatment with tetrabutyalmmonium (TBA^+), tetrapentylammonium (TPA^+), or tetraoctylammonium (TOA^+) ions, each at 3 µM or with N^G-nitro-L-arginine methyl ester (L-NAME) at 100 µM. L-arginine at 1 mM antagonized the inhibitory effect of TBA^+ and L-NAME, but not of TPA^+ and TOA^+. TPA^+ and TOA^+, but not TBA^+, also inhibited endothelium-independent relaxation induced by a NO donor, hydroxylamine. The inhibitory effect of TPA^+ was absent in 50 mM K^+-containing Krebs solution. These results indicate that (1) TBA^+ inhibits endothelial NO-mediated relaxation probably through inhibition of NO production and/or release; (2) TPA^+-induced inhibition of endothelial and exogenous NO-dependent relaxation may be mediated through blockade of K^+ channels in aortic smooth muscle; and (3) TOA^+ may act on both endothelium and smooth muscle to inhibit NO-mediated vasorelaxant effect.

Introduction

Monovalent quaternary ammonium ions have been useful pharmacological tools in ion channel research. These agents block various types of K^+ channels in a diversity of cells with varying levels of effectiveness [1]. For example, a series of quaternary ammonium ions, when applied extracellularly, blocked large-conductance Ca^{2+}-activated K^+ (BK) channels. Changes in the side-chain length alter the apparent K^+ channel blocking potency [2]. We previously characterized the stoichiometry, kinetics, and voltage dependence of the blocking action of tetraethylammonium ions (TEA^+) in vascular smooth muscle cells. External TEA^+ produced a flickery block of BK channels. The concentration dependence for reduction in mean unitary current was consistent with 1:1 blinding, with dissociation constants (K_d) in the rat and rabbit arterial smooth muscle cells of 196 and 159 µM, respectively, at the membrane holding potential of 0 mV [3]. These values were close to that (K_d=250 µM) for TEA^+ in blocking the macroscopic BK current in the isolated human coronary artery smooth muscle cells [4]. TEA^+ constricted the pressurized rabbit cerebral artery through blocking BK channels [5]. TPA^+ prevented the vasorelaxation induced by K^+ channel openers [6]. TPA^+ also blocked vascular BK channels and internal TPA^+ (K_d=101 µM) showed much higher blocking potency than external TPA^+ (K_d=1.49 mM) [3,7]. In intact vessels, TPA^+ induced dual effects, constriction at concentrations smaller than 10 µM and relaxation at concentrations greater than 10 µM; the latter is K^+ channel-independent [8].

The role of K^+ channels in the regulation of the membrane potential and Ca^{2+} homeostasis in the endothelium is well recognized. Blockade of K^+ channels in non-excitable endothelial cells would cause membrane depolarization and thus narrows the driving force for Ca^{2+} influx. This action may partly explain the inhibitory effects of some quaternary ammonium ions on endothelial NO-mediated

vasorelaxation [9,10]. TBA^+ was found to inhibit cyclic GMP-independent vasorelaxation induced by interaction of thiols with NO stores [11]. TOA^+ produced a transient relaxation that was sensitive to inhibitors of the NO-dependent dilators [12]. In this report, we described the effects of three monovalent quaternary ammonium ions (TBA^+, TPA^+ and TOA^+) with varied side-chain length on both endothelial and exogenous NO-mediated relaxation in the rat aorta and to test whether L-arginine, the NO precursor could antagonize the inhibitory effects on endothelial NO-dependent aortic relaxation.

Materials and Methods

Male Sprague-Dawley rats (~ 250-300 g) were killed by cervical dislocation. The thoracic aorta was excised and three 3 mm-wide ring segments were prepared from each aorta. Each ring was mounted between two stainless wire hooks in a 10-ml organ bath filled with Krebs solution of the following composition (mM): 119 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 1.2 KH_2PO_4, and 11 D-glucose. The bath solution was gassed with 95% O_2 and 5% CO_2, and kept at 37 ^oC (pH ≈7.4). The rings were placed under an optimal basal tone of 15 mN and changes in isometric tension were measured by Grass force transducer. In some rings the endothelial layer was mechanically disrupted by rubbing the luminal surface of a ring with plastic tubing. Endothelium removal was confirmed by the failure of the rings to relax in response to 1 μM acetylcholine.

Once a steady contraction was induced by U46619, CPA was added cumulatively to produce concentration-dependent relaxations in endothelium-intact rings or hydroxylamine added to produce endothelium-independent relaxation in endothelium-denuded rings. In another set of experiments, the rings were exposed to each quaternary ammonium ion or L-NAME 30 min prior to addition of U46619, the effect of CPA was examined. In some experiments, the rings were first exposed to 1 mM L-arginine for 10 min prior to addition of the inhibitors; the relaxant effect of CPA was then tested. The effects of three ammonium ions were also tested on the endothelium-independent relaxation induced by hydroxylamine, a NO donor in endothelium-denuded rings. The initial tone was made comparable in amplitude by adjusting the concentration of U46619.

The following drugs were used: phenylephrine, acetylcholine, CPA, TBA^+, TPA^+, TOA^+, ODQ, L-arginine, L-NAME (from Sigma or RBI).

The relaxant effects were presented as percentage reduction of agonist-induced tone. Concentration-response curves were analyzed by non-linear curve fitting using Graphpad software. The negative logarithm of the dilator concentration that produced 50% the maximum relaxation (pD_2) and the maximal response (E_{max}) were calculated. For statistical analysis, Student's *t*-test was used and statistical difference was accepted when $P < 0.05$. The results are means ± SEM of *n* rings.

Results

Effect on endothelial nitric oxide-mediated relaxation

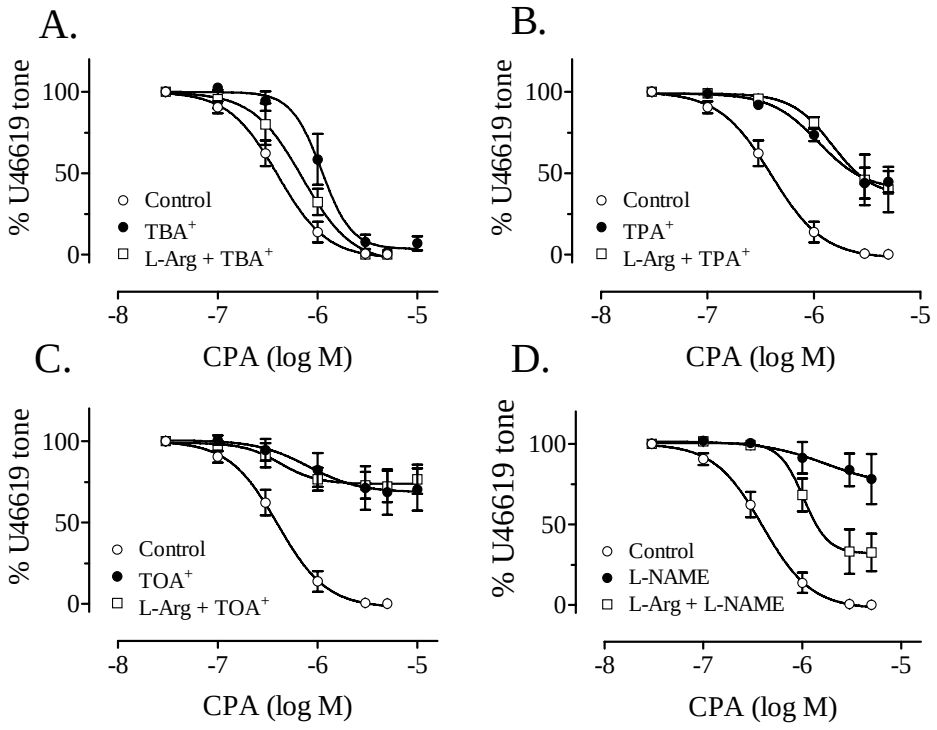
Fig. 1 shows the inhibitory effects of TBA^+, TPA^+ and TOA^+, each at 3 μM on CPA-induced relaxation in endothelium-intact rat aortic rings. CPA, an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase induced concentration-dependent relaxations with a pD_2 of 6.40 ± 0.06 (n=5, Fig. 2). The pD_2 values for CPA-induced effect were 5.90 ± 0.05, 5.96 ± 0.11, and 6.10 ± 0.36 in the presence of TBA^+, TPA^+ and TOA^+, respectively (Fig. 2). The rank order of inhibition potency is $TOA^+ > TPA^+ > TBA^+$ (Fig. 2). Both TPA^+ and TOA^+ reduced the maximal relaxation induced by CPA, while TBA^+ had no effect (Fig. 2B). The relaxant response to CPA was reduced by 100 μM L-NAME and abolished in endothelium-denuded rings (n=4-5).

Effect of L-arginine

Fig. 1 also shows the effect of 1 mM L-arginine on attenuated CPA-induced relaxation in the presence of various blockers. L-Arginine did not affect the inhibitory effects of TPA^+ or TOA^+ on CPA-induced relaxation (Fig. 1B-C). In contrast, L-arginine significantly antagonized the effect of TBA^+ or L-NAME (Fig. 1A and D).

Effect on exogenous nitric oxide-mediated relaxation

Treatment with TBA^+ did not affect hydroxylamine-induced relaxation in U46619-preconstricted rings (Fig. 3A). In contrast, both TPA^+ and TOA^+ attenuated the endothelium-independent relaxant response to hydroxylamine (Fig. 3A). The inhibitory effect of TPA^+ was abolished in 50 mM K^+-contacted rings, while TOA^+ was still able to reduce the relaxant effect of hydroxylamine in the high K^+ solution (Fig. 3D). Again, TBA^+ did not influence hydroxylamine-induced relaxation of high K^+-contracted rings (Fig. 3D). The pD_2 and E_{max} values for hydroxylamine-induced relaxation were summarized in Fig. 3B-C in U46619-contracted rings and in Fig. E-F in 50 mM K^+-contracted rings. ODQ (a selective inhibitor of guanylate cyclase) at 3 μM abolished the hydroxylamine-induced relaxation while L-NAME at 100 μM had no effect (n=4-5), thus confirming the NO-mediated cGMP-dependent relaxation in response to hydroxylamine.



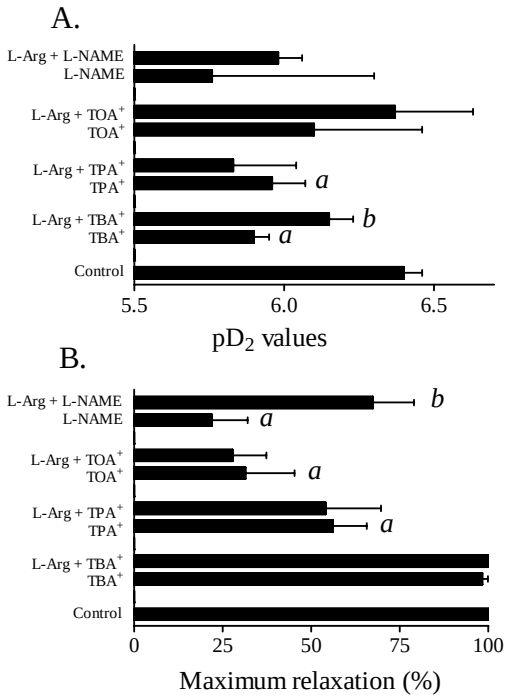


Fig. 1: *Log concentration-response curves for CPA-induced effect in control and in the presence of TBA^+ (A), TPA^+ (B), TOA^+ (C) each at 3 µM or L-NAME at 100 µM (D). Effect of L-arginine (1 mM) on inhibition of CPA-induced relaxation induced by TBA^+ (A), TPA^+ (B), TOA^+ (C) or L-NAME (D). These experiments were performed in the endothelium-intact rings preconstricted by U46619. Data are means ± SEM of 4-5 experiments.*

Fig. 2: *The pD_2 (A) and E_{max} (B) values obtained after various pharmacological interventions described in Fig. 1. Data are means ± SEM of 4-5 experiments. a indicates difference from the control group and b indicates difference between ammonium ion group and L-arginine-treated group ($P < 0.05$).*

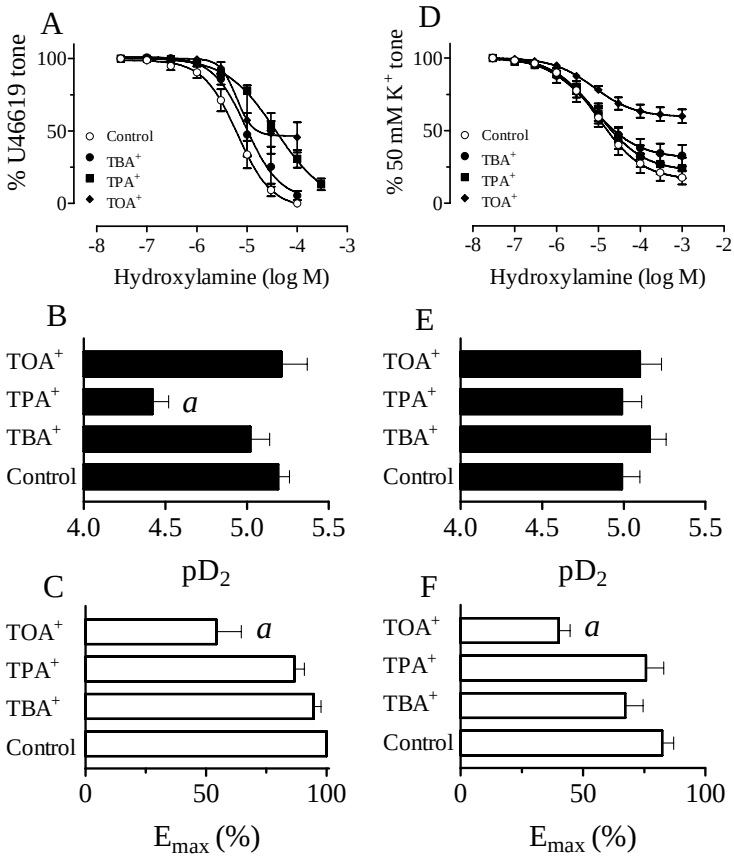


Fig. 3: *Effects of TBA^+, TPA^+, or TOA^+ each at 3 µM on hydroxylamine-induced relaxation in endothelium-denuded rings preconstricted by U46619 (A) with pD_2 (B) and E_{max} (C) values or 50 mM K^+ (D)) with pD_2 (E) and E_{max} (F) values. Data are means ± SEM of 4-5 experiments. a indicates difference from control ($P < 0.05$).*

Discussion

The present results show the inhibitory effects of TBA^+, TPA^+ and TOA^+ on endothelial NO-mediated relaxation in the isolated rat aortic rings. The rank order of inhibition effectiveness is $TOA^+ > TPA^+ > TBA^+$. It is at present unknown whether the potency of inhibiting endothelial NO-mediated relaxation is related to the length of the side-chain on the methylene group among the three quaternary ammonium ions. TOA^+ and L-NAME (a known inhibitor of NO synthase, NOS) reduced CPA-induced relaxation to the similar degree, but the concentration of TOA^+ (3 µM) is over 30-fold lower than L-NAME (100 µM). Treatment with L-arginine, the precursor of NO biosynthesis partially antagonized the inhibitory effect on endothelium/NO-dependent relaxation, which was induced by L-NAME or TBA^+ but not by TPA^+ or TOA^+. This indicates that TBA^+ may act via mechanisms that are different from those mediating the effects of TPA^+ and TOA^+. It can not be ruled out that TBA^+ may competitively inhibit endothelial NOS as does L-NAME.

Once produced in the endothelial cells, NO readily crosses the endothelial cell membrane and diffuses towards the underlying smooth muscle cells to activate guanylate cyclase, a key enzyme that triggers NO-mediated aortic relaxation. In order to examine whether quaternary ammonium ions could inhibit guanylate cyclase as a main mechanism by which endothelial NO-dependent relaxation is inhibited, the effects of TBA^+, TPA^+ and TOA^+ were tested on the endothelium-independent relaxation induced by an exogenous NO donor. TPA^+ and TOA^+, each at 3 µM significantly inhibited the relaxations induced by hydroxylamine, a NO donor in the endothelium-denuded rings [13]. In contrast, TBA^+ was without an effect. This again indicates that the mechanisms of action may be different between TBA^+ and TPA^+ or TOA^+ when used in the same concentration range. It is possible that both TPA^+ and TOA^+ may interfere with any steps along a chemical cascade starting from guanylate cyclase in vascular smooth muscle.

Many quaternary ammonium ions are pharmacological blockers of vascular K^+ channels [3,7]. NO was found

to activate the vascular Ca^{2+}-activated K^+ channels [14]. It is, therefore, possible that part of ammonium ions-induced effect on NO-dependent relaxation is mediated through inhibition of K^+ channels on aortic smooth muscle cells. The inhibitory effect of TPA^+ was lost in the aortic rings preconstricted by 50 mM K^+. One of the principal effects of raising extracellular K^+ concentration is to decrease the electrochemical gradient for K^+ efflux, so that the influence of K^+ channel activation by NO donors or inhibition by quaternary ammonium ions would have been blunted. These results indicate that TPA^+-induced inhibition of both endogenous and exogenous NO-mediated relaxation may be mediated via inhibition of K^+ channels. However, TOA^+ still attenuated NO donor-induced relaxation, indicating more complex actions on blood vessels.

In summary, TBA^+, TPA^+, and TOA^+ all reduced endothelial NO-mediated relaxation but via different mechanisms. TBA^+ may act partly as an inhibitor of NO production in the endothelium and TPA^+ may act as a putative blocker of cGMP-dependent K^+ channels in vascular smooth muscle. Whilst, TOA^+ acts in a more complex manner, which may target both endothelium and vascular smooth muscle leading to inhibition of NO-dependent aortic relaxation. In view of the complexity of the vascular actions, caution must be taken when employing quaternary ammonium ions even at their low concentrations as pharmacological tools in endothelium-intact blood vessels.

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